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WPI Acc No: 94-249973/199431

Medicaments contg. steroid(s), e.g. *sersasapogenin* - useful in treatment of e.g. tumours, viral infections, *Alzheimer*'s disease, psoriasis and diabetes

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Number of Countries: 001 Number of Patents: 001
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
DE 4303214	A1	19940811	DE 4303214	A	19930204	A61K-031/58	199431 B

Priority Applications (No Type Date): DE 4303214 A 19930204
Patent Details:

Patent	Kind	Lan	Pg	Filing Notes	Application	Patent
DE 4303214	A1		12			

Abstract (Basic): DE 4303214 A

Medicaments contg. one or more steroids of formulae (Ia)-(Ie) are new. In (Ia): RA = OH and RB = a gp. of formula (i); in (Ib) RA+RB = a gp. of formula (ii); in (Ic) RA+RB = a gp. of formula (iii); in (Id) RA+RB = a gp. of formula (iv); in (Ie) RA = H and RB = a gp. of formula (v); X = O (in cpds. (Ia)-(d)) or NR9 (in cpds. (Ie)); Rc = O, H, OH or NH2; RD = Me; RE = Me or CH2OH; RF = Me, CH2OH or CH2. The substit.

R is always in the beta-configuration; the substits. R8, R11, R13 and R14 are always in the alpha-configuration; The rings B/C and C/D are always trans and the rings D/E are always cis; The rings A/B can be cis-(5beta, R4) or trans-(5alpha, R4) linked; A double bond may exist between C4 and C5, C5 and C6, C12 and C13 and C13 and C14; The configuration at C22 and C25 can be R or S;
R1,R2,R3,R4,R5,R6,R9,R10,R12,R16,R17,R18,R19 = H, OH or amino, in the alpha- or beta-configuration; R8,R11,R13,R14 = H, OH or amino in the alpha-configuration; or R0 is absent when a double bond exists between C12 and C13 or C13 and C14; in this case R14 can be H or Me; When Ring A is aromatic, substits. R4 and R0 are absent; In this case R1 and R3 can be HOCH2 oe Me; R7,R15 = H, OH or amino in the beta-configuration; R1,R2,R3,R4,R%,R6,R9,R10,R12,R16,R17,R19 = an oxo gp.. Each hydroxy or amino gp. may be glycosidated with a sugar, alkylated with an alcohol or acyclated with an acid.

Pref. the prefd. cpd. (I) is 3-beta-hydroxy, 5-beta-H, 255-spirostan (*sarsasapogenin*) (I').

. USE - The medicaments are useful in treatment of disorders viral, viroidal or oncogene origin. These include viral infections (esp. HIV) autoimmune disorders, chronic inflammatory and

inflammatory-degenerative processes, tumours or neoplastic or pathogen-proferative processes. Specific disorders include leukaemia, lung cancer, prostate cercinoma, Parkinson syndrome, Alzehimer's disease, arthritis, rheumatism, gout, astma, insulin-dependent Diabetes, dermatitis, psoriasis. Spondylitis ankylopeotica, etc.. Admin. is esp. oral, rectal, intraveous or intramuscular. Dosage of (I) is 0.5-15 mg/kg/day.

Dwg.0/0

Derwent Class: B01

International Patent Class (Main): A61K-031/58

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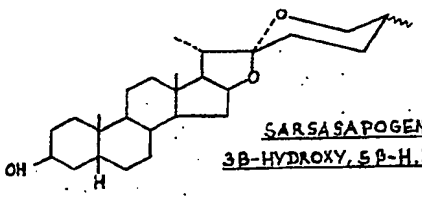
gleich Anmelder

(54) Behandlung von Erkrankungen viraler, viroidaler oder onkogener Genese durch Steroid-Saponine oder deren Aglykone

(57) Arzneimittel zur Behandlung von Erkrankungen viraler, viroidaler oder onkogener Genese enthaltend eine oder mehrere Verbindungen mit Furostan-, Spirostan-, Furo-Spirostan-, Spirosolan- oder Solanidin-Skelett. Das Beispiel zeigt einen Weg zur Behandlung von HIV-Infektionen und Prostata-Carcinomen durch Sarsasapogenin (3β-Hydroxy-5β,25S Spirostan).



SARSASAPOGENIN
3β-HYDROXY, 5β-H, 25S-SPIROSTAN

DE 43 03 214 A1

Der Erfinder hat die Funktion von UsnRNA und Leader, die Funktion der Polyadenylierung sowie der bereits erwähnten sekundären HRPs entdeckt und das Zusammenwirken dieser und anderer Faktoren beim Processing von Primärtranskripten beschrieben.

Der Erfinder hat weiter entdeckt, daß es außer den in der Literatur erwähnten caps eine große Zahl weiterer caps gibt, die sich sowohl in Zahl und Struktur der Basen (es gibt nicht nur 1-, 2-, und 3-basige, sondern auch 4-basige caps), als auch in der Methylierung der cap-Basen unterscheiden.

Bei der Untersuchung von caps in ein- und mehrzelligen tierischen Organismen hat der Erfinder festgestellt, daß es offensichtlich eine phylogenetische Entwicklung gegeben hat von den einfachen 1-basigen (Guanin)-caps über die 2- und 3-basigen bis zu den 4-basigen caps, wie man sie nur in hochentwickelten tierischen (und menschlichen) Zellen findet. Auch hat er festgestellt, daß die Anfügung eines sogenannten poly-A-Schwanzes (Anfügen von poly-Adenylsäure-Resten an das Primärtranskript) in diskreten, also definierten Größenordnungen geschieht und daß definierte Stellen des poly-A-Schwanzes spezifische Methylierungen aufweisen.

Von besonderem Interesse im Zusammenhang mit dieser Erfindung ist dabei die Tatsache, daß sich Viren offenbar bei ihrer Replikation in menschlichen Zellen einiger spezieller, phylogenetisch "junger" cap-Strukturen bedienen. Diese virustypischen cap-Strukturen werden (Ontogenese reproduziert die Phylogenese) im menschlichen Organismus dementsprechend entweder nur in den frühesten Stadien der Entwicklung oder nur in einigen wenigen Zellen des adulten Organismus gebildet, die noch auf einer relativ "niedrigen" Entwicklungs- bzw. Differenzierungsstufe stehen. Die gleichen cap-Strukturen hat der Erfinder auch bei Primärtranskripten von Onkogenen und proteinogenen Viroiden sowie bei viroidaler RNA identifiziert.

Weiter ist im Zusammenhang mit dieser Erfindung von Interesse, daß die Verbindung eines Leaders und einer homogenen oder heterogenen RNA immer durch eine spezifische UsnRNA katalysiert wird, die — wie bereits erwähnt — an einer markanten Stelle eine Komplementär-Sequenz zu der Consensus-Sequenz des Leaders aufweist. Diese UsnRNA nämlich bringt das 3'-Ende des Leaders und das 5'-Ende der zu verbindenden homogenen RNA bzw. das erste Exon der heterogenen RNA in eine Position, die es einer wiederum spezifischen Ligase ermöglicht, Leader und homogene RNA bzw. Exon miteinander zu verbinden.

Der Erfinder hat zweifelsfrei nachweisen können, daß die stereochemische Struktur des Spleissosoms und die in ihm ablaufenden Reaktionen durch die bereits weiter oben erwähnten sekundären HRPs katalysiert werden: ohne diese sekundären HRPs ist sowohl in vivo als auch in vitro die Bildung des Spleissosoms und damit die Synthese einer reifen mRNA unmöglich.

Im Zuge seiner Arbeit über die der Genexpression und dem Processing zugrundeliegenden biochemischen Prozesse ist der Erfinder auch der Frage nachgegangen, welcher biochemischen Funktion Viroide ("nackte RNA-Mini-Viren") ihre Pathogenität verdanken. Während man bei Pflanzen verschiedene Viroide identifiziert und ihre Wirkungen auf die Pflanze beschrieben hat, ist die Pathogenität von Viroiden in der Human-Medizin noch Gegenstand der Diskussion. Man ist sich noch nicht einmal darüber einig, ob die RNA von Viroiden für Proteine kodiert oder nicht.

Der Erfinder hat nun entdeckt, daß Viroide entweder für (Hormon-Rezeptor)-Proteine kodieren oder ihre Pathogenität der Tatsache verdanken, daß ihre RNA-Sequenz mit der eines RNA-Leaders oder einer UsnRNA identisch ist. Die humanpathogenen Wirkungen von Viroiden beruhen also entweder auf der Beeinflussung der Genexpression oder der des Processing. Diese Beobachtungen, die im Detail noch der Überprüfung und Bestätigung bedürfen, könnten nicht nur die sogenannten slow-virus-Infektionen erklären helfen, sondern auch Erklärungen bieten für eine ganze Reihe von Krankheiten, deren virale oder viroidale Genese noch in der Diskussion oder deren Ätiologie noch gänzlich ungeklärt ist.

Zwischen den Funktionen von Viroiden und Onkogenen gibt es Parallelen: nach Feststellungen des Erfinders kodieren auch Onkogene zum Teil für cytoplasmatische Hormon-Rezeptorproteine, für Plasmamembran-Rezeptoren oder für Wachstums-Hormone (Growthfactors), die an solche Rezeptoren oder Rezeptorproteine binden. Auch können Onkogene für RNAs (UsnRNAs oder Leader-Sequenzen) kodieren, also nicht proteinogen und doch pathogen sein. Eine Differenzierung zwischen Onkogen und Viroid ist also im Grunde nicht durch deren Struktur oder Funktion, sondern nur durch ihre pathogenen Wirkungen in der jeweiligen Zelle möglich.

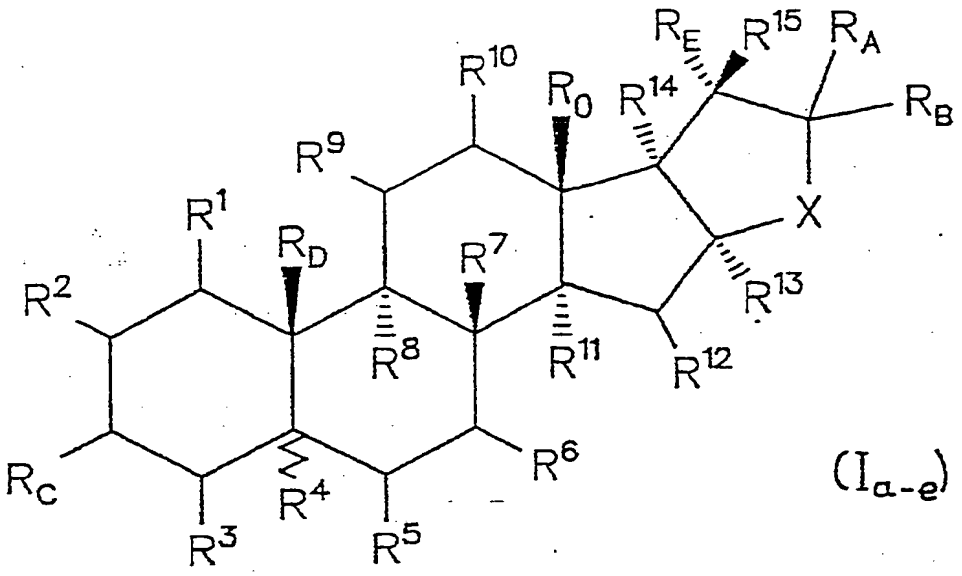
Wie eng die Verwandtschaft zwischen Viroiden oder viralen Genen und Onkogenen ist, zeigt die Tatsache, daß zwischen dem Onkogen abl — das in Mäusen die sogenannte Abelson-Leukämie verursacht — und dem Gen "tat" des humanen Immunschwäche-Virus HIV-1 eine 90%ige Sequenz-Homologie besteht.

Für die Integration von humanpathogenen Viroiden oder viralen Onkogenen ins Zellgenom kommen verschiedene Mechanismen in Betracht, die hier nicht im Detail diskutiert werden sollen: außer durch Onkornaviren werden Viroide/Onkogene mit hoher Wahrscheinlichkeit auch durch andere RNA-Viren (Retro-, Reo-, Calici-, Picorna-, Corona-, Orthomyxo-, Paramyxoviren) als Vektoren in den menschlichen Organismus eingeschleust und durch Reverse Transkription oder RNA/DNA-Hybridisierung und Plasmidbzw. Episom-Bildung ins Zellgenom inseriert.

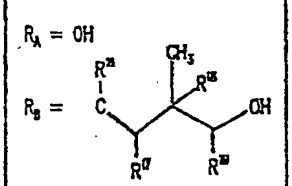
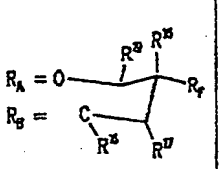
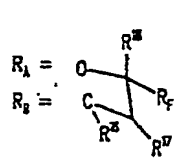
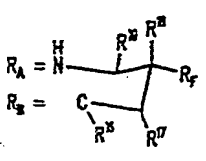
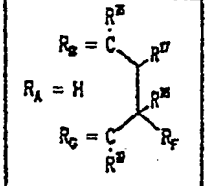
Da die Pathogenität von Viroiden oder Onkogenen nach Erkenntnissen des Erfinders also eng mit der Genexpression und dort zu einem erheblichen Teil mit den Mechanismen des Spleißprozesses korreliert ist, ist die Blockierung der pathogenen viroidalen oder onkogenen Mechanismen im Prinzip auf dem gleichen Wege wie bei viralen Genen möglich.

Bei seinen Untersuchungen und Experimenten zur Analyse der Vorgänge im Spleissosom hat der Erfinder sich verschiedener Naturstoffe bedient, um die einzelnen Stufen des Processing in bestimmten Stadien wirksam unterbrechen und untersuchen zu können.

Dabei hat er entdeckt, daß eine Reihe von steroidalen und den Steroiden verwandten Naturstoffen, die in pflanzlichen, zum Teil aber auch in tierischen Organismen an einer hormonellen oder hormon-analogen Steuerung der Transkription und der Verarbeitung der Transkripte beteiligt sind, in Abhängigkeit von Struktur und Methylierung des caps und der Methylierung einer definierten Zahl von Basen am 5'-Ende des Leaders, von Zahl



(I a-e)

	Furostane (a)	Spirostane (b)	Furo-Spirostane (c)	Spirosolane (d)	Solanidine (e)
					
X =	O	O	O	O	N-R_C
R_C =	O, H, OH, NH_2	O, OH, H, NH_2	O, H, OH, NH_2	O, H, OH, NH_2	O, OH, H, NH_2
R_D =	CH_3	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	———	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

für die allgemein gilt,
daß der Substituent R7 stets β-ständig, die Substituenten R8, R11, R13 und R14 stets α-ständig sind — die Ringe B/C und C/D also stets trans- und die Ringe D/E stets cis-verknüpft sind. Die Ringe A/B können sowohl cis- (5β-R4) als auch trans-verknüpft (5α-R4) sein. Zwischen C4 und C5, C5 und C6, C12 und C13 sowie zwischen C13 und C14 kann eine Doppelbindung vorliegen. Die Konfiguration an C22 und C25 kann jeweils R oder S sein.

Weiter gilt:

Die Substituenten R1, R2, R3, R4, R5, R6, R9, R10, R12, R16, R17, R18 und R19 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder eine Amino-Gruppe in α- oder β-Stellung sein.

R8, R11, R13 und R14 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder Amino-Gruppe in α-Stellung sein. Wenn zwischen C12 und C13 oder C13 und C14 eine Doppelbindung vorliegt, entfällt R0. R14 kann dann eine Methylgruppe oder ein H-Atom sein.

Wenn der Ring A aromatisch ist, entfallen die Substituenten R4 und RD. R1 und R3 können dann unabhängig voneinander eine Methyl- oder Hydroxymethylgruppe sein.

R7 und R15 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder eine Amino-Gruppe in β-Stellung,
R1, R2, R3, R5, R6, R9, R10, R12, R16, R17 und R19 unabhängig voneinander eine Oxogruppe sein.

Außerdem gilt, daß Hydroxy- oder Amino-Gruppe mit einem Zucker glykosidiert, mit einem Alkohol alkyliert oder mit einer Säure acyliert sein kann.

Zum zweiten einen Weg zur Behandlung von Erkrankungen viraler, viroidaler und onkogener Genese durch Verwendung eines Arzneimittels, dessen aktiver Bestandteil eine Substanz der allgemeinen Formel (Ia—e) ist
zum dritten einen Weg zur Behandlung von Erkrankungen viraler, viroidaler und onkogener Genese durch

ve, neoplastische und/oder pathogen-proliferative Prozesse sowie Krankheiten ein, die durch Onkogene verursacht werden.

Zu den Krankheiten, die mit einem erfindungsgemäßen Arzneimittel behandelt werden können, gehören insbesondere Infektionen mit

Retro-Viridae (alle HIV-Serotypen, HTLV I und HTLV II), Oncornaviridae, Herpes-Viridae (Alpha-, Beta- und Gammaherpesviren), Parvoviridae, Pox- und Parapoxviridae, Picornaviridae (alle Rhinoviren, Cardioviren, Coxsackie A und B, Echoviren, Enteroviren (Hepatitis A), Hepatitis-B-Virus und Delta-Agens, Polio I, II, III, Calici-Viridae, Orbiviridae, Rubiviridae, Orthomyxoviridae (Influenza A, B, C), Paramyxoviridae (Parainfluenza, Mumps), Bunyaviridae, Arenaviridae, NANB-Hepatitis-Viren, Norwalk-, Ebola- und Marburg-Viren.

Zu den Krankheiten, die nach Erkenntnissen des Erfinders direkt oder indirekt durch Viren und/oder Viroide verursacht werden und deshalb mit einem erfindungsgemäßen Arzneimittel behandelt werden können, gehören z. B.

das Parkinson-Syndrom, die Alzheimersche Erkrankung, Arthrosen und Arthritiden/Gicht, Erkrankungen des rheumatischen Formenkreises, Asthma, die Nephropathia epidemica, Multiple Sklerose, der insulinabhängige Diabetes mellitus, Neuritiden, Dermatiden, Auto-Immunkrankheiten und die Psoriasis.

Weiter gehören zu den Krankheiten, die nach den Erkenntnissen des Erfinders mit einem erfindungsgemäßen Arzneimittel behandelt werden können benigne und maligne Tumore, insbesondere des Magen-Darm-Trakts, der Lungen, des Gehirns, der Haut und des Genitale (insbesondere Prostata-Karzinome und -sarkome, Zervix- und Mammakarzinome, Blasenhalsadenome), aber auch pathogen proliferative oder neoplastische Prozesse wie Leukämien, Erythrämien und Erythro-Leukämien.

Ein erfindungsgemäßes Arzneimittel kann in gelöster Form oder in Form einer pharmazeutischen Zubereitung intravenös, intramuskulär, oral und rektal verabreicht werden oder auch für die externe Anwendung (z. B. bei durch Herpes-simplex verursachten Läsionen) zu Salben, Cremes, Pudern, Lotions, Ölen oder Emulsionen verarbeitet werden. Für die orale Anwendung kommen insbesondere Tabletten oder Kapseln — auch magensaft-resistente — in Frage. Für die Injektion oder Infusion kommen die bekannten Lösungs- und Aufbereitungsverfahren zur Anwendung.

Die wirksame Dosis eines erfindungsgemäßen Arzneimittels hängt außer von der jeweils verwendeten spezifischen Substanz nach Formel (Ia—e) von einer Reihe weiterer Faktoren ab, wie z. B. Art und Schwere der Erkrankung, Allgemeinzustand und Alter des Behandelten sowie — bei HIV-Infektionen/AIDS zum Beispiel — von Art und Schwere der assoziierten Infektionen und Erkrankungen. Im allgemeinen dürfte die Dosis bei interner Anwendung pro kg/ pro Tag zwischen 0,5 mg und 15 mg und damit etwa in der Größenordnung bakterieller Antibiotika liegen. Die wirksame Dosis kann in Einzelfällen aber auch wesentlich über oder unter dieser Dosis liegen. Die Gesamtdosis kann auf 2 bis 6 Gaben pro Tag verteilt werden.

Bei externer Anwendung sollte die Konzentration der Substanz nach Formel (Ia—e) zwischen 50 und 1000 Mikrogramm (0,05 bis 1 mg) pro Gramm Arzneimittel-Grundlage betragen.

Im Folgenden wird an Hand von Beispielen die Wirkung einer Substanz nach Formel (Ia—e) aufgezeigt.

Beispiel 1:

Eine chronisch HIV-infizierte CD4+ T-Zell-Linie (MOLT-4, ATCC CRL 1582, J. Minowada, Roswell Park Memorial Institute, Buffalo, New York) wurde über einen Zeitraum von 7 Tagen in Anwesenheit von Sarsasapogenin (3β-Hydroxy-5β,25S Spirostan) kultiviert.

Kontroll-Kulturen wurden während des gleichen Zeitraums mit Lösungsmittel behandelt.

Die Zellen wuchsen in 50 cm^3-Kulturflaschen in 5 ml eines RPMI 1640-Mediums mit einem Zusatz von 10% fetalem Kälber-Serum (FCS) bei 37° Celsius und 5% CO_2-Begasung.

Während die zu therapierenden Kulturen in Abständen von etwa 6 Stunden mit 200 µl (entsprechend 26 µg Sarsasapogenin pro ml Medium) einer Stocklösung (0,65 mg Sarsasapogenin in 1 ml 45%igem Glycerol gelöst) behandelt wurden, erhielten die Kontrollen die gleiche Menge 45%iges Glycerol.

Um eine Anreicherung des Lösungsmittels in den Kulturen zu vermeiden, wurden die Zellen vor jedem Nachdosieren mit 1000 rpm fünf Minuten zentrifugiert, das alte Medium verworfen und das Pellet in frischem Medium aufgenommen.

Nach 7 Tagen (gleich 28 Behandlungsschritten) wurden Proben der Kulturüberstände für den HIV-1 p24 Core-Profile ELISA-Test (DU PONT Nen) entnommen, gemäß Originalprotokoll verarbeitet und die jeweiligen-Gehalte an p24 ermittelt.

Beispiel 2:

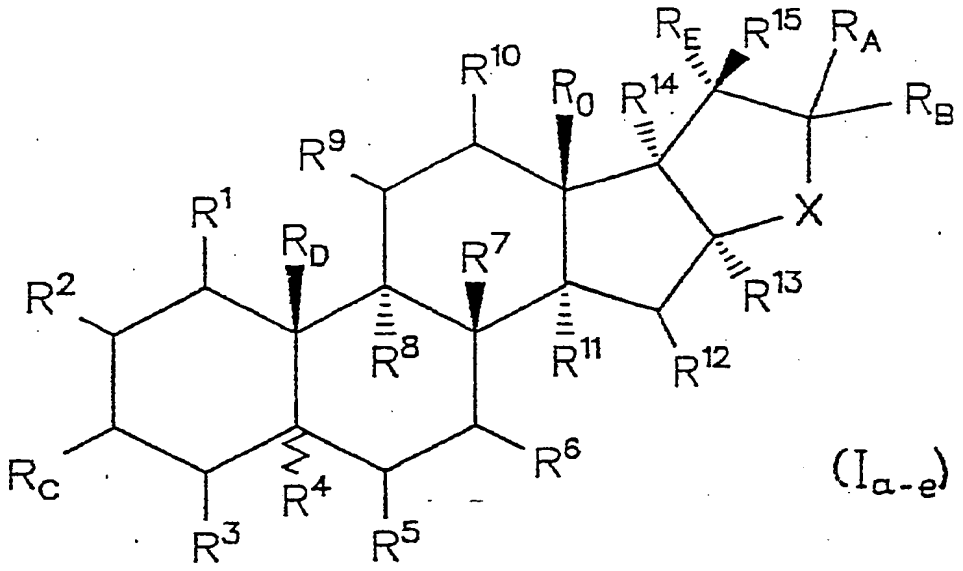
Eine chronisch HIV-infizierte CD4+ T-Zell-Linie (MOLT-4, ATCC CRL 1582, J. Minowada, Roswell Park Memorial Institute, Buffalo, New York) wurde über einen Zeitraum von 7 Tagen in Anwesenheit von Sarsasapogenin (3β-Hydroxy-5β,25S Spirostan) kultiviert.

Kontroll-Kulturen wurden während des gleichen Zeitraums mit Lösungsmittel behandelt.

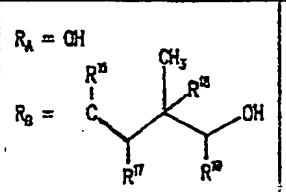
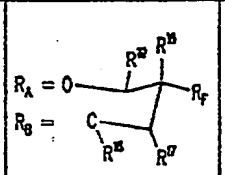
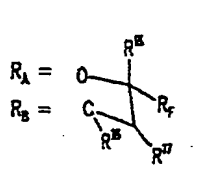
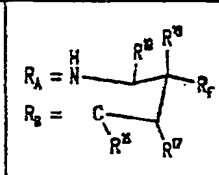
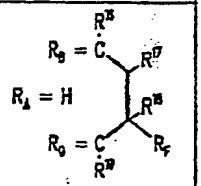
Die Zellen wuchsen in 50 cm^3-Kulturflaschen in 5 ml eines RPMI 1640-Mediums mit einem Zusatz von 10% fetalem Kälber-Serum (FCS) bei 37° Celsius und 5% CO_2-Begasung.

Während die zu therapierenden Kulturen in Abständen von 12 Stunden mit 200 µl (entsprechend 26 µg Sarsasapogenin pro ml Medium) einer Stocklösung (0,65 mg Sarsasapogenin in 1 ml 45%igem Glycerol gelöst) behandelt wurden, erhielten die Kontrollen die gleiche Menge 45%iges Glycerol.

Um eine Anreicherung des Lösungsmittels in den Kulturen zu vermeiden, wurden die Zellen vor jedem Nachdosieren mit 1000 rpm fünf Minuten zentrifugiert, das alte Medium verworfen und das Pellet in frischem

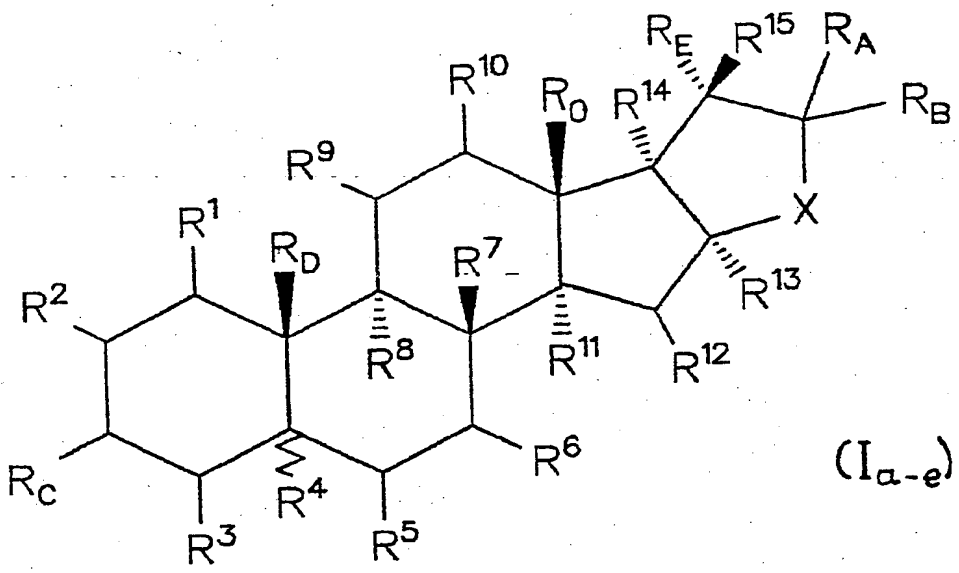


(I a-e)

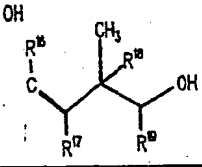
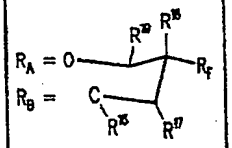
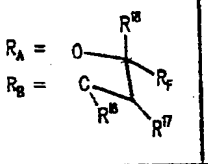
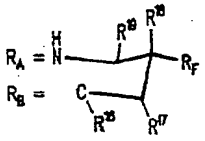
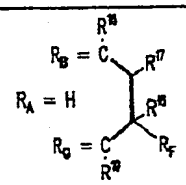
	Furostane (a)	Spirostane (b)	Furo-Spirostane (c)	Spirosolane (d)	Solanidine (e)
					
X =	O	O	O	O	N–R_C
R_C =	O, H, OH, NH_2	O, OH, H, NH_2	O, H, OH, NH_2	O, H, OH, NH_2	O, OH, H, NH_2
R_O =	CH_3	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	———	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

für die allgemein gilt,
daß der Substituent R7 stets β-ständig, die Substituenten R8, R11, R13 und R14 stets α-ständig sind — die Ringe B/C und C/D also stets trans- und die Ringe D/E stets cis-verknüpft sind. Die Ringe A/B können sowohl cis- (5β-R4) als auch trans-verknüpft (5α-R4) sein. Zwischen C4 und C5, C5 und C6, C12 und C13 sowie zwischen C13 und C14 kann eine Doppelbindung vorliegen. Die Konfiguration an C22 und C25 kann jeweils R oder S sein.
Weiter gilt:
Die Substituenten R1, R2, R3, R4, R5, R6, R9, R10, R12, R16, R17, R18 und R19 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder eine Amino-Gruppe in α- oder β-Stellung sein.
R8, R11, R13 und R14 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder Amino-Gruppe in α-Stellung sein. Wenn zwischen C12 und C13 oder C13 und C14 eine Doppelbindung vorliegt, entfällt R0. R14 kann dann eine Methylgruppe oder ein H-Atom sein.
Wenn der Ring A aromatisch ist, entfallen die Substituenten R4 und RD. R1 und R3 können dann unabhängig voneinander eine Methyl- oder Hydroxymethylgruppe sein.
R7 und R15 können unabhängig voneinander ein H-Atom, eine Hydroxy- oder eine Amino-Gruppe in β-Stellung
R1, R2, R3, R5, R6, R9, R10, R12, R16, R17 und R19 unabhängig voneinander eine Oxogruppe sein.
Außerdem gilt, daß jede Hydroxy- oder Amino-Gruppe mit einem Zucker glykosidiert, mit einem Alkohol alkyliert oder mit einer Säure acyliert sein kann.
2. Arzneimittel enthaltend eine oder mehrere Verbindungen der allgemeinen Formel (Ia).
3. Arzneimittel enthaltend eine oder mehrere Verbindungen der allgemeinen Formel (Ib).

Nummer: DE 43 03 214 A1
Int. Cl.[5]: A 61 K 31/58
Offenlegungstag: 11. August 1994



(I_{a-e})

	Furostane (a)	Spirostane (b)	Furo-Spirostane (c)	Spirosolane (d)	Solanidine (e)
R_A =	OH	O	O	H, N	H
R_B =					
X =	O	O	O	O	N–R_C
R_C =	O, H, OH, NH_2	O, OH, H, NH_2	O, H, OH, NH_2	O, H, OH, NH_2	O, OH, H, NH_2
R_D =	CH_3	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	———	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

ZEICHNUNG (1)

11/8/94 P48

DE 4303214 A1 (English translation)

Description

The present invention describes a way of treating diseases that are caused by viruses, viroids or oncogenes, with steroid saponins having a furostane, spirostane, furo-spirostane, spirosolane or solanidine skeleton or their aglucons.

Virus infections have increasingly become one of the most widespread forms of disease, and not just since the discovery of HIV, and one of the most dangerous and least accessible forms of disease on account of their causal treatment. This is due on the one hand to the fact that little is known about the mechanisms of transcription and the processing of transcripts in human cells, and on the other hand that in vitro conditions can be transferred only with difficulty to physiological processes in the human body.

At the present time only a few virostatics are employed in clinical practice: 5-iodo-2'-desoxyuridine is used to treat keratitis, which is caused by the herpes simplex virus or vaccinia virus - N-methyl-beta-thiosemicarbazone is used prophylactically and therapeutically in variola type diseases and in vaccinia gangrenosa and generalised vaccinia. 1-adamantanamine acts against specific viruses that cause colds and related infections, e.g. against influenza A viruses.

Various agents have been used for some time to treat HIV infections, some of which admittedly specifically inhibit the progression of the disease, at least for a certain period, but have serious side effects (Aciclovir, AZT), while others act non-specifically and do not attack the causative agents, but instead assist the immune system in its defensive role.

Glycirrhizin has been described as antivirally effective in vitro against HIV (Antiviral Research, 7 (1987), 127 - 137), although clinical tests unfortunately have not been very encouraging.

A European patent application (EP 0 442 744 A2) describes the treatment of virus infections with cardenolides and bufadienolides ("cardioactive glycosides"). The applicants and inventors show that these substances are able in the cell fusion test to inhibit the formation of giant cells in cell cultures infected with the herpes simplex virus. The applicants do not know, or at least have not described, the action mechanism.

It is also significant that the tests described in the application were carried out with glycocidic natural substances -a treatment in vivo might well be problematic on account of the size of the requisite doses and the known low therapeutic spectrum of the cardioactive glycosides.

It is undisputed that viral genes (oncogenes) can lead to a malignant transformation of a cell. Particularly in the case of RNA tumour viruses, i.e. oncornaviruses, but also in the case of DNA tumour viruses, a large number of genes have been found that are associated with tumours or pathogenically proliferative processes.

The mechanism of tumour formation itself is unknown. It is however virtually certain that the, without exception, strongly preserved gene products of oncogenesis have a decisive role in controlling the proliferation and differentiation of cells.

A whole range of viruses have been implicated in malignant tumours in humans, although an aetiological

connection between a virus infection and the malignant tumour has been confirmed only in a very small number of cases. For example, infection with the hepatitis B virus can lead to the formation of a hepato-cellular carcinoma, while the HTLV I and II viruses (human T cell leukaemia virus), which are classed as retroviruses, can cause leukaemia. The human immunodeficiency virus HIV, which has only recently been discovered and identified, has definitely been implicated in a number of tumours, but is probably aetiologically connected with their occurrence only by blocking the body's own cellular cytolytic mechanisms.

The inventor has been investigating for many years the mechanisms of gene expression in human cells and has been concerned in particular with the processes involved in the conversion of primary transcripts into mature mRNA. In this connection the inventor has on the one hand investigated the interactions between activated primary HRPs (complexes formed from a core membrane-receptor protein and a releasing hormone) and histone proteins in gene activation, and on the other hand has investigated the biochemical role of such hormones that are induced by releasing hormones. Activated complexes formed from these messenger substances (termed by the inventor secondary transcription hormones) and cytoplasmic receptor proteins (secondary HRPs) play an important role in the processing (processing/splicing) of primary transcripts in the splicosome. The inventor has investigated in detail the processes occurring in gene activation as well as the processing of primary transcripts, and has largely identified and characterised the factors involved in this.

The inventor has discovered inter alia with reference to the splicing process (processing) and the relevant processes within the scope of this invention,

that a large part of the repetitive sequences of the human genome represents leader sequences;
that these leader sequences exhibit homologies (consensus sequences), and can thus be combined to form leader groups having identical consensus sequences (leader codes);
that in each case there is a specific UsnRNA for these leader codes (to which also the known CAAT and the TATA box belong), which has a complementary sequence to a leader code;
that leader codes and UsnRNAs can be associated with specific hormones;
that a fully formed mRNA is always produced by the joining (splicing) of two parallel transcribable primary transcripts, one of which represents a leader sequence and the other represents the primary transcript of the gene (a homogeneous or heterogeneous RNA).

Depending on whether the primary transcript represents a homogeneous (monocistronic) or heterogeneous (polycistronic) hnRNA the transcripts of leader sequence and gene are then processed further. The already known procedure of differential splicing of hnRNA leads in various cell populations or in various differentiation stages of the same cell line, to differently structured mRNAs and thus to different gene products.

The inventor has discovered the function of UsnRNA and leaders, the function of polyadenylation, as well as of the already mentioned secondary HRPs, and has described the interaction of these and other factors in the processing of primary transcripts.

The inventor has furthermore discovered that, apart from the caps mentioned in the literature, there is a large number of further caps that differ both in the number and structure of the bases (there are not only 1-,2- and

3-base caps, but also 4-base caps), as well as in the methylation of the cap bases.

In the investigation of caps in single-cell and multicellular animal organisms the inventor has found that there is obviously a phylogenetic development from the simple 1-base (guanine) caps, via the 2- and 3-base caps, to the 4-base caps, such as are found only in highly developed animal (and human) cells. The inventor has also established that the attachment of a so-called poly-A tail (attachment of polyadenylic acid residues to the primary transcript) occurs in discrete and thus defined orders of magnitude and that specific sites of the poly-A tail exhibit specific methylations.

Of particular interest in connection with this invention is the fact that viruses clearly utilise some special, phylogenetically "young" cap structures during their replication in human cells. These virus-typical cap structures (ontogenesis reproduces the phylogenesis) are accordingly formed in the human body either only in the earliest stages of development or in only a few cells of the adult organism, which are still at a relatively "low" stage of development or differentiation. The inventor has identified the same cap structures also in primary transcripts of oncogenes and proteinogene viroids as well as in viroidal RNA.

It is also of interest in connection with this invention that the joining of a leader and a homogeneous or heterogeneous RNA is always catalysed by a specific UsnRNA which - as already mentioned - exhibits at a prominent site a complementary sequence to the consensus sequence of the leader. This UsnRNA in fact brings the 3'-end of the leader and the 5'-end of the homogeneous RNA to be joined and/or the first exon of the heterogeneous RNA into a position that in turn enables a

specific ligase to join leader and homogeneous RNA and/or exon to one another.

The inventor has undoubtedly been able to show that the stereochemical structure of the splicosome and the reactions occurring therein are catalysed by the aforementioned secondary HRPs; without these secondary HRPs the formation of the splicosome and thus the synthesis of a fully formed mRNA is impossible in vivo as well as in vitro.

In the course of his work on gene expression and the fundamental biochemical processes involved in the processing, the inventor has also dealt with the question of the biochemical function that is responsible for the pathogenecity of viroids ("naked RNA mini-viruses"). Whereas various viroids have been identified in plants and their effects on plants have been described, the pathogenecity of viroids in human medicine is still the subject of discussion. There is still no clear agreement as to whether the RNA of viroids does or does not code for proteins.

The inventor has now discovered that viroids code either for (hormone-receptor) proteins, or that their pathogenecity is due to the fact that their RNA sequence is identical to that of a RNA leader or of a UsnRNA. The human pathogenic effects of viruses are thus due either to their influencing of gene expression or of processing. These observations, which still have to be checked and confirmed in detail, have been able to explain not only the so-called slow virus infections, but also provide an explanation for a whole number of diseases whose viral or viroidal origin is still under discussion or whose aetiology is still completely unknown.

There are parallels between the functions of viroids and oncogenes: according to the inventor's results oncogenes also to some extent code for cytoplasmic hormone receptor proteins, for plasma membrane receptors or for growth hormones (growth factors), which bind to such receptors or receptor proteins. Oncogenes may also code for RNAs (UsnRNAs or leader sequences), i.e. although not proteinogenic they are nevertheless pathogenic. It is thus in principle possible to differentiate between oncogenes and viroids not through their structure or function, but only through their pathogenic effects in the relevant cell.

The closeness of the relationship between viroids or viral genes and oncogenes is shown by the fact that there is a 90% sequence homology between the oncogene abl - which in mice causes so-called Abelson's leukaemia - and the gene "tat" of the human immunodeficiency virus HIV-1.

Various mechanisms may be used to integrate human pathogenic viroids or viral oncogenes into the cell genome, which will not be discussed in more detail here: viroids/oncogenes are inserted with a high degree of probability as vectors into the human body by, apart from oncornaviruses, also by other RNA viruses (retro, reo, calici, picorna, corona, orthomyxo, paramyxoviruses) and are inserted into the cell genome by reverse transcription or RNA/DNA hybridization and plasmid or episome formation.

Since the pathogenicity of viroids or oncogenes is in the opinion of and according to the inventor's knowledge thus closely correlated with the gene expression, which in turn is correlated to a considerable extent with the mechanisms of the splicing process, the pathogenic

viroidal or oncogenic mechanisms may in principle be blocked in the same way as with viral genes.

In his investigations and experiments on the analysis of the processes occurring in the splicosome, the inventor has utilised various natural substances in order to be able effectively to interrupt and investigate the individual processing steps at specific stages.

The inventor has discovered that a number of steroidal substances and natural substances related to steroids that in plant organisms and to some extent also in animal organisms are involved in a hormonal or hormone-like control of the transcription and processing of the transcripts are able, depending on the structure and methylation of the cap and the methylation of a specific number of bases at the 5'-end of the leader, and on the number and methylation of the poly-A residue and the complementary sequence of the cap UsnRNA, to inhibit just as well or even better in human cells, very specifically the processing of homogeneous and heterogeneous (monocistronic and polycistronic) viral and viroidal/oncogenic RNA.

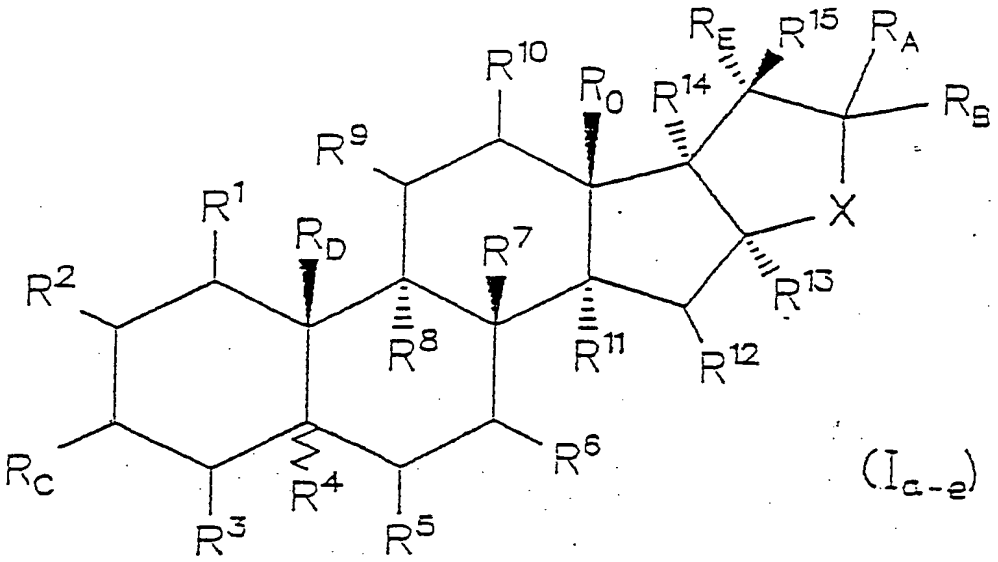
The natural substances forming the basis of this patent application, which have a fairly large affinity for the binding sites of the splicosome, displace the secondary HRPs (phylogenesis dominates the oncogenesis) from their binding sites and thus prevent the formation of the function-oriented conformation of the splicosome.

Under the influence of the aforementioned natural substances the synthesis of specific viral mRNAs, the synthesis of non-physiological pathogenic proteins coded by viroids or cellular oncogenic genes (growth factors, receptor proteins) and the pathogenic effects of

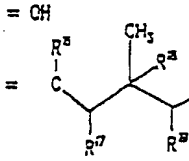
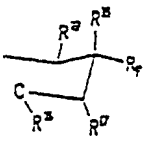
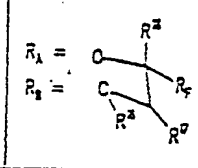
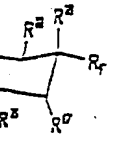
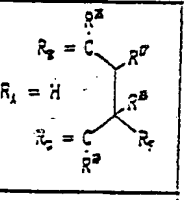
oncogenically coded RNA or viroid-RNA (false UsnRNAs, false leaders) are inhibited both in vitro and in vivo.

Up to now the etiology of a large number of diseases in humans and animals has been unclear. Such diseases include not only conditions such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, certain leukaemias and erythremas, most neoplasias or kuru and scrapie, in which a viral or subviral and/or viroidal or oncogenic etiology is already more or less being seriously discussed, but in particular also the chronic inflammatory or degenerative diseases of the postural and locomotor system (rheumatic diseases, arthroses and arthritis/gout), autoimmune diseases, as well as insulin-dependent diabetes and psoriasis, in which up to now scarcely anyone has considered that they might be caused by viruses or viroids. The inventor is however convinced that a considerable proportion of these diseases and illnesses of unknown or unclear origin are caused by viruses and/or by subviral units (viroids/ oncogenes) imported by the latter, and furthermore that these diseases can be causally treated by means of the natural substances disclosed in his patent application.

The present invention describes firstly a way of treating diseases of viral, viroidal and oncogenic origin by administration of the effective amount of a substance of the general formulae (Ia-e)



(I a-e)

Furostanes (a)		Spirostanes (b)	Furo-Spirostanes (c)	Spirosolanes (d)	Solanidines (e)
R_A = OH R_B = 		R_A = O R_B = 	R_A = R_B = 	R_A = NH R_B = 	R_B =  R_A = H R_C =
X =	O	O	O	O	N–R_E
R_C =	O, H, OH, NH_2	O, OH, H, NH_2	O, H, OH, NH_2	O, H, OH, NH_2	O, OH, H, NH_2
R_D =	CH_3	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	———	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

for which it is generally true that, the substituent R7 is always in the beta-position, the substituents R8, R11, R13 and R14 are always in the alpha-position - the rings B/C and C/D are thus always trans-coupled and the rings D/E are always cis-coupled. The rings A/B may be both cis-coupled (5beta-R4) and trans-coupled (5alpha-R4). There may be a double bond between C4 and C5, C5 and C6, C12 and C13, as well as

between C13 and C14. The configuration of C22 and C25 may in each case be R or S.

It is furthermore true that:

The substituents R1, R2, R3, R4, R5, R6, R9, R10, R12, R16, R17, R18 and R19 may independently of one another be a H-atom, of a hydroxy or amino group in the alpha or beta position.

R8, R11, R13 and R14 may independently of one another be a H-atom, a hydroxy or amino group in the alpha position. If there is a double bond between C12 and C13 or C13 and C14, R0 is absent and R14 may then be a methyl group or a H-atom.

If the ring A is aromatic, the substituents R4 and RD are absent, and R1 and R3 may then independently of one another be a methyl or hydroxymethyl group.

R7 and R15 may independently of one another be a H-atom, a hydroxy or an amino group in the beta position.

R1, R2, R3, R5, R6, R9, R10, R12, R16, R17 and R19 may independently of one another be an oxo group.

It is also true that the hydroxy or amino group may be glycosided with a sugar, alkylated with an alcohol, or acylated with an acid.

The invention secondly describes a way of treating diseases of viral, viroidal and oncogenic origin by use of a drug whose active constituent is a substance of the general formulae (Ia-e), and thirdly describes a way of treating diseases of viral, viroidal and oncogenic origin by using a drug whose active constituent comprises several substances of the general formulae (Ia-e) in an arbitrary mixing ratio, and fourthly the

invention describes a way of treating diseases of viral, viroidal and oncogenic origin by use of a drug whose active constituent is one or more substances of the general formulae (Ia-e) in an arbitrary mixing ratio, in conjunction with additives, auxiliaries, excipients and vehicles, solvents and/or solubility promoters, as are conventional or possible in galenic pharmacy.

It is generally known that many of the steroid saponins with which the present invention is involved contain a common aglucon (genin, sapogenin), and thus differ only in the form, composition and bonding of the sugars. It is also generally recognised that the physiologically active group in steroid saponins (e.g. in the cardioactive glycocides), i.e. also compounds of the formulae (Ia-e), is the aglucon or genin. The substances that are to be protected by this patent application are characterised by the fact that they contain a furostane, spirostane, furo-spirostane, spirosolane or solanidine skeleton.

Typical examples of the substances on which the invention is based are the sapogenins alliogenin, agigenin, 2-0-Ac-epimetagenin, barogenin, chlorogenin, convallagenin A and B, convallamarogenin, demissidin, digalogenin, digitogenin, diosgenin, eduligenin, epidiosgenin, episceptrumgenin, epiruscogenin, gentrogenin, gitogenin, hecogenin, heloniogenin, hispigenin, igagenin, isocarneagenin, isonarthogenin, isoplexigenin, isoreinekkiagenin, isorhodeasapogenin, isorubijervin, isojurubidin, jurubidin, karatavegenin, kitogenin, kogagenin, kryptogenin, laxogenin, leptinidin, lowegenin, luvigenin, manogenin, markogenin, metagenin, meteogenin, mexogenin, neoagigenin, neoalliogenin, neochlorogenin, neogitogenin, neonogiragenin, neotigogenin, neotokorogenin,

nogiragenin, nologenin, nuatigenin, paniculogenin, pennogenin, protometagenin, reineckiagenin, rhodeasapogenin, rockogenin, rubijervin, ruscogenin, samogenin, sarsasapogenin, sisalagenin, smilagenin (neosarsasapogenin), soladulcidin, soladunalinidin, solagenin, solanaviol, solasodenon, solasodin, solasonin, trillenogenin, tigogenin, tokorogenin, tomatidin, veramin, yonogenin, yuccagenin, yamogenin and their respective glycosides.

The substances according to formulae (Ia-e) can be obtained either by extraction and purification from natural sources (e.g. plants of the families Liliaceae, Amaryllidaceae, Smilacaceae, Cactaceae, Trilliaceae, Discoreaceae, Balanitaceae, Agavaceae, Zygophyllaceae, Solanaceae, Ruscaceae, Scrophulariaceae or Ranunculaceae) or by generally known chemical processes involving condensation of an aglucon with a physiologically compatible group (sugar, alkyl or acyl radical).

Modifications on one or more arbitrary C atoms that instead of a H-atom, a hydroxy, amino, carbonyl group, or a glycosided, alkylated or acylated hydroxy amino group, are provided with another physiologically compatible group, have only an indirect influence on the action of the drug, namely only the manner and speed of its resorption. This means that the substances or compounds that are to be protected by this patent application also include those derivatives and substrates whose use in the meaning of this invention leads by metabolic processes in the organism to compounds of the general formulae (Ia-e).

The term sugar group should be understood in its widest sense. The sugars mentioned hereinafter should

therefore be regarded only as examples and not as an exhaustive list.

By the term sugar are understood monosaccharides, oligosaccharides and polysaccharides, which may be linear or branched. Typical sugars are for example glucose, galactose, rhamnose, xylose, pyranose, quinovose, apiose, arabinose, furanose, L-fucose, mannose, timobiose, chacotriose, lycotetraose or digitopentaose. The term sugar or sugar group also includes the relevant isomeric and anomeric forms as well as modifications of the sugar molecules.

As acyl groups there may be mentioned in particular organic carboxylic acids belonging to the aliphatic, cycloaliphatic, aromatic, aromatic-aliphatic or heterocyclic series, for example formic acid, acidic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, onanthic acid, caprylic acid, pelargonic acid, capric acid, undecanoic acid, lauric acid, trimethylacetic acid, tert. butylacetic acid, cyclopentylacetic acid, diethylaminoacetic acid, morpholinoacetic acid, lactic acid, succinic acid, adipic acid, benzoic acid and nicotinic acid.

Suitable inorganic acids include, inter alia, sulphuric or phosphoric acid.

The esters of some acids may optionally be converted with alkali into the water-soluble salts.

Suitable alkyl groups are derived from the alcohols of the corresponding organic acids.

The term "treatment" used in this application includes all forms of treating diseases of viral, viroidal and

oncogenic origin, in particular the prevention, prophylaxis, control, improvement and cure.

The expression "diseases of viral, viroidal or oncogenic origin" refers to the ability of the substances forming the basis of the patent application, in the human body or animal organisms

a) to inhibit or completely suppress the synthesis of viral mRNA and thus the multiplication (replication) of viruses, as well as
b) to inhibit or completely suppress the synthesis of pathogenic mRNAs that is effected by viroids or oncogenes, as well as
c) to inhibit or completely suppress the pathogenic effects of viroids or oncogenes that are caused by mismanagement of mechanisms of gene expression or processing.

By analogy with what was mentioned above, this therefore also includes chronic inflammatory and inflammatory degenerative, neoplastic and/or pathogenic-proliferative processes as well as diseases that are caused by oncogenes.

The diseases that may be treated with a drug according to the invention include in particular:
Retroviruses (all HIV serotypes, HTLV I and HTLV II), oncornaviruses, herpes viruses, (alpha, beta and gamma herpes viruses), parvoviruses, poxviruses and parapoxviruses, picornaviruses (all rhinoviruses, cardioviruses, coxsackie A and B viruses, echoviruses, enteroviruses (hepatitis A), hepatitis B virus and delta agent, polio I, II, III, caliciviruses, orbiviruses, rubiviruses, orthomyoxyviruses (influenza A, B, C), paramyoxyviruses (parainfluenza, mumps), bunyaviruses,

arenaviruses, NANB hepatitis viruses, Norwalk, Ebola and Marburg viruses.

Among the diseases that in the experience of the inventor are directly or indirectly caused by viruses and/or viroids and that can therefore be treated with a drug according to the invention, are for example the following:
Parkinson's disease, Alzheimer's disease, arthroses and arthritis/gout, rheumatic diseases, asthma, nephropathia epidemica, multiple sclerosis, insulin-dependent diabetes mellitus, neuritis, dermatitis, autoimmune diseases, and psoriasis.

Diseases that in the experience of the inventor can be treated with a drug according to the invention furthermore include benign and malignant tumours, especially of the gastro-intestinal tract, lungs, brain, skin and genitals (especially prostate carcinoma and sarcoma, cervical cancer and breast cancer, bladder cervix adenoma), and also pathogenically proliferative or neoplastic processes such as leukaemias, erythremas and erythro-leukaemias.

A drug according to the invention may be administered in dissolved form or in the form of a pharmaceutical preparation, intravenously, intramuscularly, orally and rectally, or also, in the case of external application (e.g. to treat lesions caused by herpes simplex), in the form of ointments, creams, powders, lotions, oils or emulsions. Tablets or capsules - also resistant to gastric juices - are particularly suitable for oral administration. The known methods of dissolution and preparation are suitable for administration by injection or infusion.

The effective dose of a drug according to the invention depends, apart from the specific substance according to formulae (Ia-e) used in any particular case, on a number of other factors such as the nature and severity of the disease, general condition and age of the patient, as well as - in the case of HIV infections/AIDS for example - on the nature and severity of the associated infections and illnesses. In general the dose for internal administration could be between 0.5 mg and 15 mg per kg per day, and thus roughly of the same order of magnitude as for bacterial antibiotics. The effective dose may in individual cases also be significantly higher or lower than this dose. The total dose may be divided over 2 to 6 administrations per day.

In the case of external application the concentration of the substance according to formulae (Ia-e) should be between 50 and 1000 micrograms (0.05 to 1 mg) per gram of drug base.

The action of a substance according to formulae (Ia-e) is illustrated hereinafter with the aid of examples.

Example 1:

A chronically HIV-infected $CD4^+$ T cell line (MOLT-4, ATCC CRL 1582, J. Minowada, Roswell Park Memorial Institute, Buffalo, New York) was cultivated over a period of 7 days in the presence of sarsasapogenin (3beta-hydroxy-5beta,25S spirostane).

Control cultures were treated with solvent throughout the whole period.

The cells were grown in 50 cm^3 culture flasks in 5 ml of a RPMI 1640 medium with an addition of 10% foetal calf serum (FCS) at 37°C and 5% CO_2 gassing.

Whereas the cultures to be subjected to the therapy were treated at roughly 6-hourly intervals with 200µl (corresponding to 26 µg of sarsasapogenin per ml of medium) of a stock solution (0.65 mg of sarsasapogenin dissolved in 1 ml of 45% glycerol), the controls received the same amount of 45% glycerol.

In order to avoid an accumulation of the solvent in the cultures, the cells were centrifuged for 5 minutes at 1000 rpm before each successive dosing, the old medium was discarded, and the pellet was taken up in fresh medium.

After 7 days (equal to 28 treatment steps) samples of the culture supernatants were taken for the HIV-1 p24 core profile ELISA test (DU PONT Nen), processed according to the original protocol, and the respective p24 contents were determined.

Example 2:

A chronically HIV-infected $CD4^+$ T cell line (MOLT-4, ATCC CRL 1582, J. Minowada, Roswell Park Memorial Institute, Buffalo, New York) was cultivated over a period of 7 days in the presence of sarsasapogenin (3beta-hydroxy-5beta,25S spirostane).

Control cultures were treated throughout the same period with solvent.

The cells were grown in 50 cm^3 culture flasks in 5 ml of a RPMI 1640 medium with an addition of 10% foetal calf serum (FCS) at 37°C and 5% CO_2 gassing.

Whereas the cultures to be subjected to the therapy were treated at roughly 12-hourly intervals with 200µl (corresponding to 26 µg of sarsasapogenin per ml of

medium) of a stock solution (0.65 mg of sarsasapogenin dissolved in 1 ml of 45% glycerol), the controls received the same amount of 45% glycerol.

In order to avoid an accumulation of the solvent in the cultures, the cells were centrifuged for 5 minutes at 1000 rpm before each successive dosing, the old medium was discarded, and the pellet was taken up in fresh medium.

After 7 days samples of the culture supernatants were taken for the HIV-1 p24 core profile ELISA test (DU PONT Nen), processed according to the original protocol, and the respective p24 contents were determined.

In both series of tests the HIV-1 p24 content in the cultures treated with sarsasapogenin was significantly reduced (in each case by 45%) compared to the cultures treated only with glycerol.

No cytotoxic effects were observed at the concentrations employed.

Although an up to 100% inhibition of the replication of the HIV would very probably have been achieved by using a different sapogenin under the chosen in vitro conditions, the inventor chose sarsasapogenin (3beta-hydroxy-5beta,25S spirostane) for the in vitro tests, since on the basis of his knowledge of the replication and splicing mechanisms he is convinced that this substance reliably prevents in vivo the replication of HIV in all cells infected or infectable by the virus.

As the example of, among others, glycirrhizin shows, in vitro results cannot always be extrapolated to in vivo conditions. Finally, the effect postulated by the inventor of a drug according to the invention can be

detected only in vivo since the selectively inhibiting action of substances of the formulae (Ia-e) on specific viral, viroidal or oncogenic mRNAs - as already mentioned above - is based on the competitive inhibition of activated complexes (HRPs) comprising a human (processing) hormone and a receptor protein. The replacement of human serum by foetal calf serum (FCS), which is done far less for reasons of cost than for reasons of the worldwide standardisation of the in vitro test systems, would have predictably affected the action of a drug according to the invention.

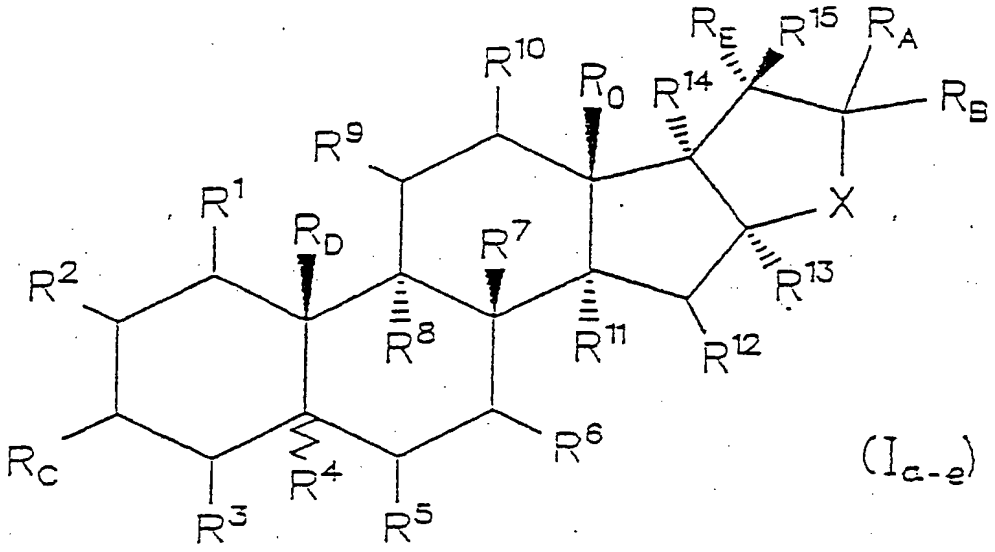
In addition, the regulation of the replication of HIV under physiological conditions (in vivo) ultimately cannot be reproduced 100% by in vitro test systems. Also, interferences or interactions between the oncogenic mechanisms of the immortalisation of the test cells and viral transcription and regulation mechanisms cannot be ruled out with certainty.

Example 3:

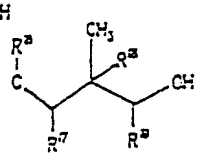
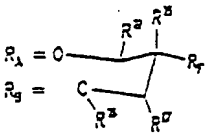
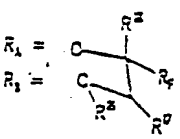
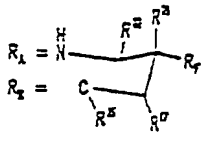
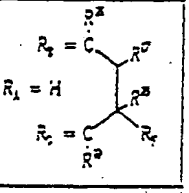
The inventor, who has for some time suffered from a prostate neoplasm and resulting micturition difficulties has - although he fully realises the uncertain outcome of such a test - carried out an experiment on himself, in which over a period of 8 weeks he self-administered sarsasapogenin, beginning with a dose of 2 x 250 mg per day up to 2 x 800 mg per day. He has observed an objective improvement in his condition: after about 14 days the micturition difficulties were almost absent and after 4 weeks had completely disappeared, and the neoplasm was no longer palpable. On the other hand the inventor did not observe any pathological reactions or changes in his condition. Also, his blood pictures were pathologically normal during the course of the experiment.

Patent Claims

1. Drug containing one or more compounds of the general formulae (Ia-e)



(Ia-e)

Furostanes (a)		Spirostanes (b)	Furo-Spirostanes (c)	Spirosolanes (d)	Solanidines (e)
R_A = CH R_B = 					
X =	O	O	O	O	N–R_E
R_C =	O, H, OH, NH_2	O, OH, H, NH_2	O, H, OH, NH_2	O, H, OH, NH_2	O, OH, H, NH_2
R_D =	CH_3	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	———	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

for which it is generally true that,
the substituent R7 is always in the beta-position, the substituents R8, R11, R13 and R14 are always in the alpha-position - the rings B/C and C/D are thus always trans-coupled and the rings D/E are always cis-coupled. The rings A/B may be both cis-coupled (5beta-R4) and trans-coupled (5alpha-R4). There may be a double bond between C4 and C5, C5 and C6, C12 and C13, as well as between C13 and C14. The configuration of C22 and C25 may in each case be R or S.

It is furthermore true that:
The substituents R1, R2, R3, R4, R5, R6, R9, R10, R12, R16, R17, R18 and R19 may independently of one another be a H atom, or a hydroxy or amino group in the alpha or beta position.

R8, R11, R13 and R14 may independently of one another be a H atom, or a hydroxy or amino group in the alpha position. If there is a double bond between C12 and C13 or C13 and C14, R0 is absent and R14 may then be a methyl group or a H atom.

If the ring A is aromatic, the substituents R4 and RD are absent, and R1 and R3 may then independently of one another be a methyl or hydroxymethyl group.

R7 and R15 may independently of one another be a H atom, or a hydroxy or an amino group in the beta position.

R1, R2, R3, R5, R6, R9, R10, R12, R16, R17 and R19 may independently of one another be an oxo group.

It is also true that the hydroxy or amino group may be glycosided with a sugar, alkylated with an alcohol, or acylated with an acid.

2. Drug containing one or more compounds of the general formula (Ia).

3. Drug containing one or more compounds of the general formula (Ib).

4. Drug according to claim 3, wherein X is an oxygen atom, RC is a hydroxy group, and RD, RE and RF are a methyl group.

5. Drug according to claim 3, wherein X is an oxygen atom, RC is a hydroxy group, RD, RE and RF are a methyl group, R4 is a H atom in the beta position, R1 to R3, R5 to R19 are in each case a H atom, and the configuration at C25 = S.

6. Drug according to claim 3, wherein X is an oxygen atom, RC is a sugar group, RD, RE and RF are a methyl group, R4 is a H atom in the beta position, R1 to R3, R5 to R19 are in each case a H atom, and the configuration at C 25 = S.

7. Drug containing sarsasapogenin.

8. Drug containing one or more compounds of the general formula (Ic).

9. Drug containing one or more compounds of the general formula (Id).

10. Drug containing one or more compounds of the general formula (Ie).

11. Drug according to claims 1 to 9 for treating diseases of viral, viroidal or oncogenic origin.

12. Drug according to claims 1 to 9 for treating virus infections, autoimmune diseases, chronic-inflammatory and chronic-degenerative processes, benign and malignant tumours, and neoplastic or pathogenic-proliferative processes.

13. Drug according to claims 1 to 9 for treating benign and malignant tumours of the gastrointestinal tract, lungs, brain, skin and genitals (prostate carcinoma and sarcoma, cervical cancer and breast cancer, bladder cervix cancer) and for treating leukaemias, erythremas and erythroleukaemias.

14. Drug according to claims 1 to 9 for treating Parkinson's disease, Alzheimer's disease, arthroses and arthritis, chronic polyarthritis, spondylitis ankylopaetica, arthrosis deformans, rheumatism, gout, asthma, nephropathia epidemica, insulin-dependent diabetes, neuritis, dermititis and psoriasis.

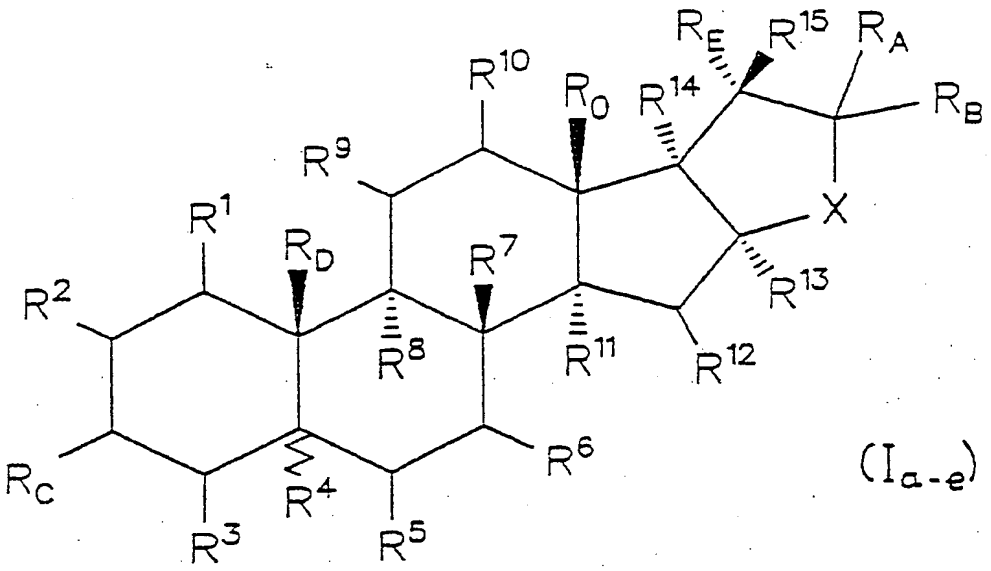
15. Drug according to claims 1 to 9 for treating prostate tumours and bladder neck adenomas.

16. Drug according to claims 1 to 9 for treating diseases caused by viruses.

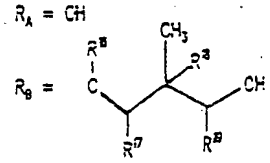
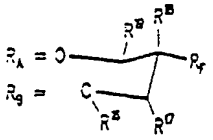
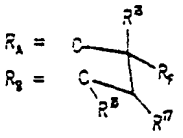
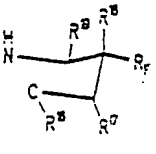
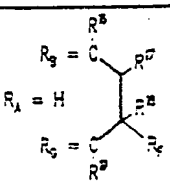
17. Drug according to claims 1 to 9 for treating diseases caused by RNA viruses.

18. Drug according to claims 1 to 9 for treating diseases caused by retroviruses and oncornaviruses.

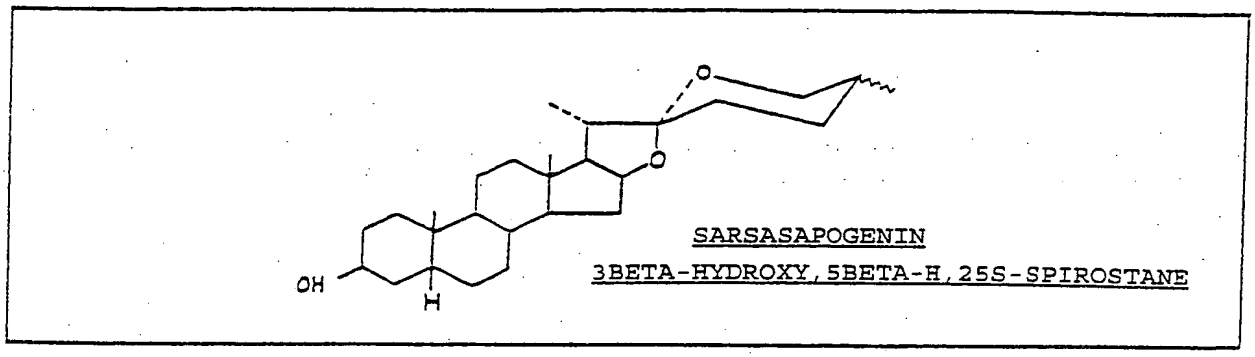
19. Drug according to claims 1 to 9 for treating diseases caused by retroviruses.



(I_{a-e})

Furostanes (a)	Spirostanes (b)	Furo-Spirostanes (c)	Spirosolanes (d)	Solanidines (e)
R_A = CH R_B = 	R_A = O R_B = 	R_A =  R_B =	R_A = NH R_B = 	R_B =  R_A = H R_C =
X =	O	O	O	N–R_C
R_C =	O, H, CH, NH_2	O, OH, H, NH_2	O, H, CH, NH_2	O, CH, H, NH_2
R_D =	CH_3	CH_3	CH_3	CH_3
R_E =	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH	CH_3, CH_2OH
R_F =	—	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$	CH_3, CH_2OH, $=CH_2$

DRAWING 1



SARSASAPOGENIN

3BETA-HYDROXY, 5BETA-H, 25S-SPIROSTANE

DRAWING 2

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